An Introduction (and more) to Primary Producers in Freshwater

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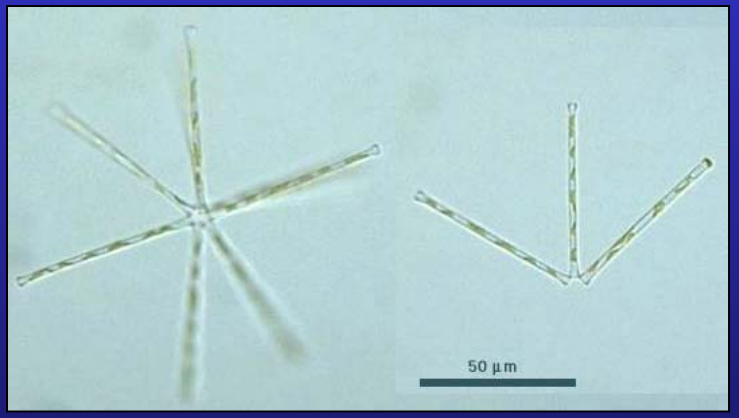
Primary Producers

- Organisms capable of converting solar energy to chemical energy
  - Phytoplankton
  - Periphyton
  - Macrophytes

Phytoplankton

- Phytoplankton: community of autotrophs adapted to suspension in the water column, which are susceptible to passive movement by wind and current.



Phytoplankton: Composition

- Common groups:
  - Chlorophytes (green algae)
  - Bacillariophytes (diatoms)
  - Cyanobacteria (blue-green algae)

Periphyton

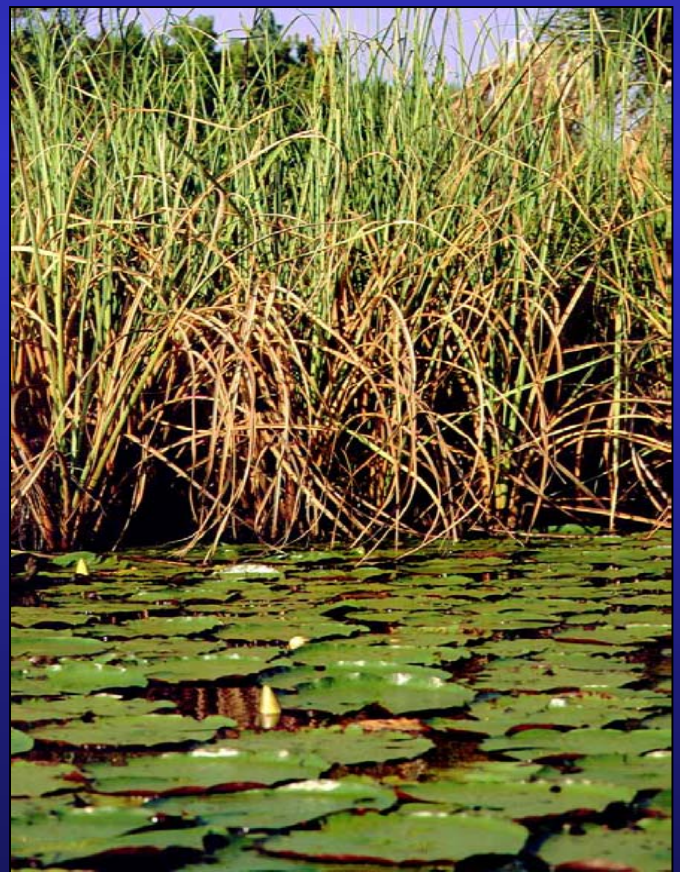
- Periphyton: assemblage of autotrophs and heterotrophs, embedded in a mucilaginous matrix, attached or floating

Periphyton: Composition

- Common groups:
  - Chlorophytes (green algae)
  - Bacillariophytes (diatoms)
  - Cyanobacteria (blue-green algae)

Macrophytes

- Macrophyte: macroscopic autotrophs, such as vascular and nonvascular plants, lichens, and large algal forms



Macrophytes: Composition

- Growth Forms:
  1. Emergents – rooted in sediments that are covered in water for at least part of the year. Nutrient uptake is almost exclusively from sediments (cattail)
  2. Attached, floating-leaved – rooted in sediments; leaves are floating. Nutrient uptake is primarily from sediments, but also from water column (*Nymphaea*)

Macrophytes: Composition

- Growth Forms:

3. Free-floating – not attached to substrate and having root or shoots in contact with water. Nutrient uptake is exclusively from water (duckweed)

4. Submerged – includes flowering plants, bryophytes, macroalgae. Rooted or attached but may detach over time; nutrient uptake: roots>leaves>stems (milfoil)

Phytoplankton: Distribution & Abundance

Habitat	Amount	Nuisance Levels - Chl a (ppb = µg/L)
Lakes/ponds/ wetlands	Abundant	15 – 20
Wadable streams	Rare	N/A
Nonwadable streams	Rare-occasionally abundant	15 – 20


Courtesy of Progressive AE

Periphyton: Distribution & Abundance

Habitat	Amount	Nuisance levels - Chl a (mg/m^2)
Lakes/ponds/ wetlands	Can be abundant in shallow areas	100 – 150
Wadable streams	Can be abundant if light is sufficient	100 – 150
Nonwadable streams	Rare-occasionally abundant	100 – 150

Macrophytes: Distribution & Abundance

Habitat	Amount	Nuisance levels DM (kg/m^2)
Lakes/ponds	Can be abundant	0.4 – 0.7 (SAV) 0.5 – 2.0 (EV)
Wetlands	Can be abundant	0.4 – 0.7 (SAV) 0.5 – 2.0 (EV)
Wadable streams	Occasionally abundant	ND
Nonwadable streams	Occasionally abundant	ND

Macrophytes: Distribution & Abundance

- Chl a poor estimator because of the large percentage of non-photosynthetic tissue in macrophytes
- Usually use dry mass for biomass
- Does sampling include both above- and below-ground biomass?

Factors Limiting Growth of Primary Producers

- Light ✓
- Grazing ✓
- Nutrients ✓
- Temperature

Light Factoids

- Sunlight is required by primary producers to photosynthesize:

$$CO_2 + 2H_2O \xrightarrow{\text{light}} (CH_2O) + H_2O + O_2$$

- Different species have different light requirements
- Usually focus on light quantity, but light quality also can be important
- Photosynthesis is highly dependent on prior light history, temperature, and dissolved inorganic carbon concentration in water

Approaches to Study Light Limitation of Primary Producers

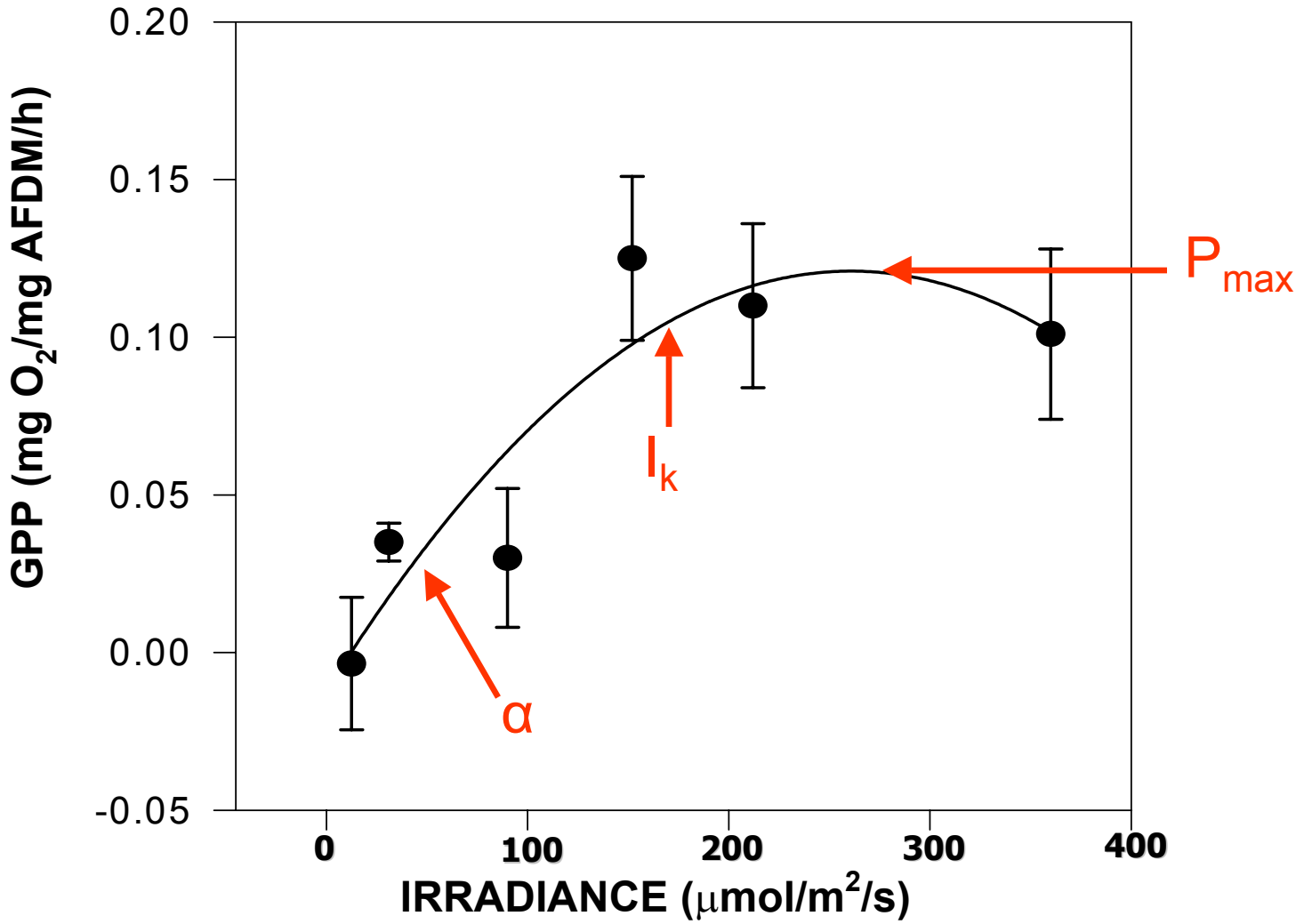
- Measure light levels in field and compare to literature values for limitation
- Measure P-I curves in the lab
- Add light; measure response variables
- Information tells you if light is limiting auto-trophic growth; if so, nutrient addition will likely not result in increased biomass or PS

Typical Values for Onset of Photosynthetic Saturation of Primary Producers

Plant Type	Irradiance ($\mu mol/m^2/s$)
Phytoplankton	20-300
Periphyton	100-400
Macrophytes	75-700

Data: Kirk (1986); Hill (1996)

P-I Curve: *Chara*



Source: Steinman et al. (1997)

P-I Parameters

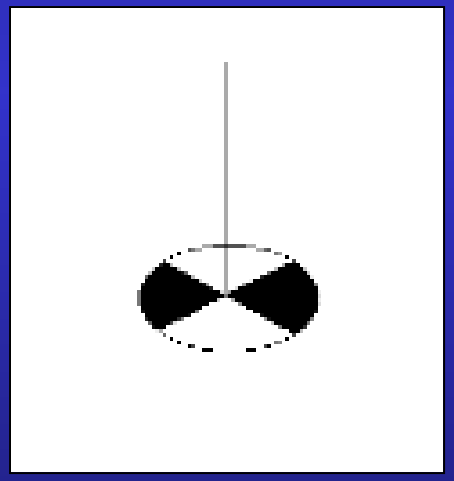
	Station	
PARAMETER	**1.2 SE**	**1.8 SE**
Water depth (m)	**2.8**	**2.3**
Irradiance (μmol m^{-2} s^{-1})	**14.5**	**45.3**
P_{max}	**0.1158**	**0.2770**
α	**0.0008**	**0.0005**
I_k	**145**	**554**

Methods to Assess Light Limitation

Method	Lakes/ ponds	Wadeable streams	Nonwade-able streams
Light levels: Secchi disk	✓	**X**	✓
Light levels: Quantum Sensor	✓	✓	✓
Light additions	**X**	✓	**X**

Secchi Disk

- 20-cm disk (usually), with alternating black and white quadrants, that measures the transparency of the water



- Transparency is affected by color of water, suspended sediments, and algae

Spring Lake, MI

Secchi Disk protocol

- Use disk of appropriate size (smaller width for shallower waters, greater width for deeper)
- Lower disk on sunny side of boat
- Allow eyes to adapt to underwater light
- Record depth at which disk disappears; raise disk and rerecord depth of reappearance; take average of 2 readings
- Water depth should be 50% greater than Secchi depth

Adapted from Davies-Colley et al. 1993

Quantum Sensor

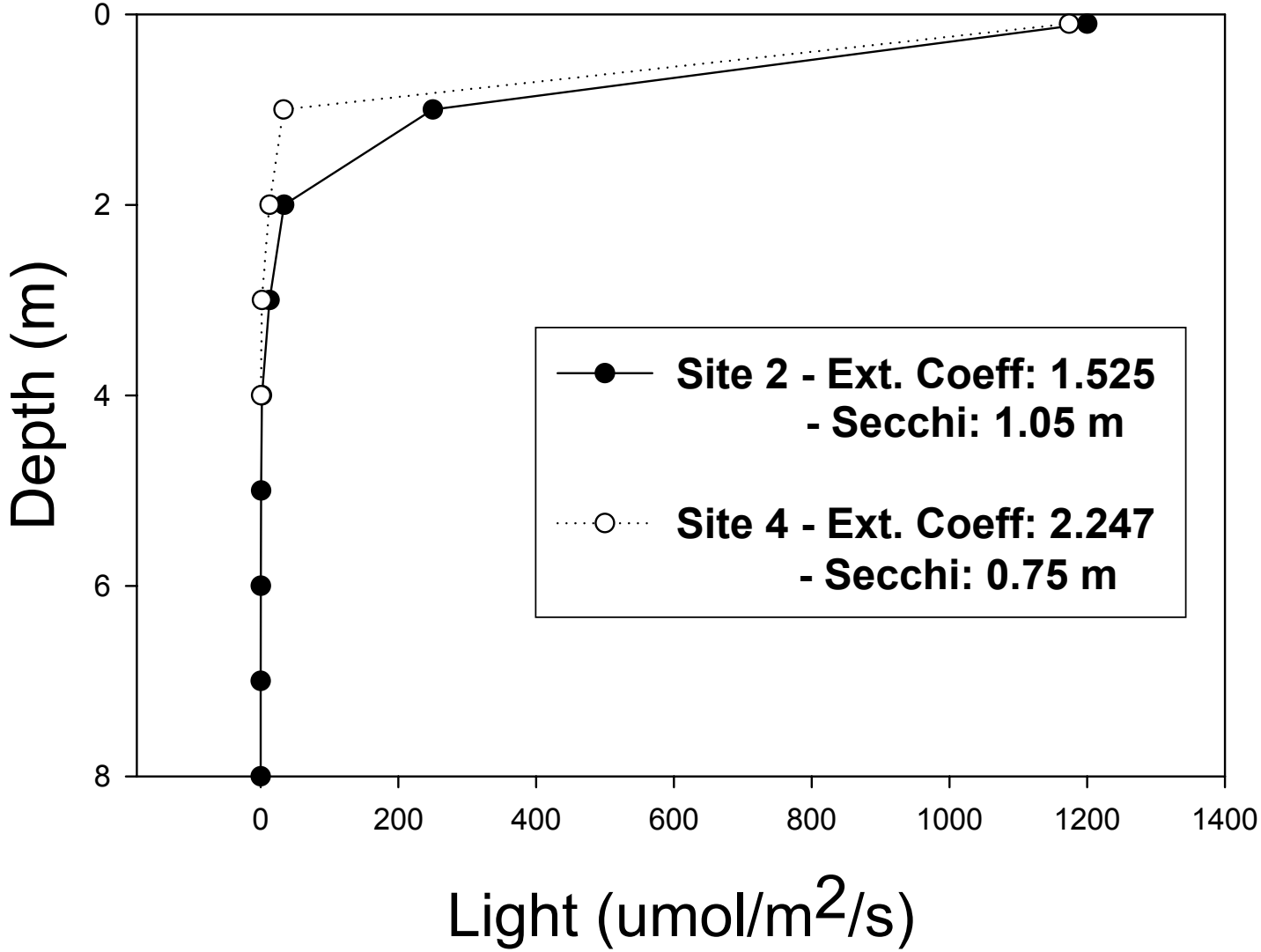
- measures photosynthetically active radiation (PAR: 400 – 700 nm)

Extinction (attenuation) Coefficient

- Due to absorption and scattering of solar radiation, the downward irradiance of the light field declines with depth
- The extinction coefficient is a measurement of vertical light attenuation (K_d)

$$K_d = \frac{1}{z_2 - z_1} \ln \frac{E_d(z_1)}{E_d(z_2)}$$

Spring Lake, MI: light profiles



Light Addition

- Add light artificially to shaded reach, *or*
- Remove canopy
- Measure response variable(s)
  - Biomass
  - Metabolism
  - Community structure

Biomass Measurements

1) Fresh mass/Dry mass (macrophytes) Ash-free dry mass (periphyton)

- gravimetric approach:
  - Fresh mass: blot dry and weigh
  - Dry mass: dry samples to constant weight
  - AFDM: oxidize dried samples in muffle furnace and reweigh oxidized samples. Loss in weight upon oxidation is AFDM

Pros: inexpensive, easy to perform

Cons: cannot distinguish algae from other organic matter (detritus, fungi); does not account for physiological state of material (senescent)

Biomass Measurements

2) Pigments (phytoplankton, periphyton)

- Spectrophotometry
  - easy to analyze, relatively inexpensive
  - requires extraction and produces waste solvents, sensitive to light, no species information

- Fluorometry
  - can be done in the field
  - more expensive, sensitive to light, no spp info.

- High performance liquid chromatography
  - very sensitive; relate to algal comm. structure
  - expensive, requires expertise, solvent waste

Biomass Measurements

3) Biovolume (phytoplankton, periphyton)

- Microscopic analysis
  - analyze subsample under microscope, measure cell morphology, and apply formulae based on cell shape to obtain biovolume

- Pros: specific to algae (avoids inclusion of other material), detailed algal community structure information

- Cons: time-consuming, requires algal taxonomic expertise, subsample must be representative, does not account for physiological state of cell

Metabolism

1) Oxygen evolution:

light ↓

$$CO_2 + 2H_2O \rightarrow (CH_2O) + H_2O + O_2$$

- measure change in oxygen over time using either chambers or whole-systems in light + dark
- Pros: accounts for physiological state of algae, integrates environmental conditions, relatively easy to do
- Cons: time-consuming; chambers may create artifacts; whole-system analysis must account for reaeration; account for respiration in light and by heterotrophs

Metabolism

2) Carbon fixation:

light ↓

$$CO_2 + 2H_2O \rightarrow (CH_2O) + H_2O + O_2$$

- measure uptake of ^{14}C from water

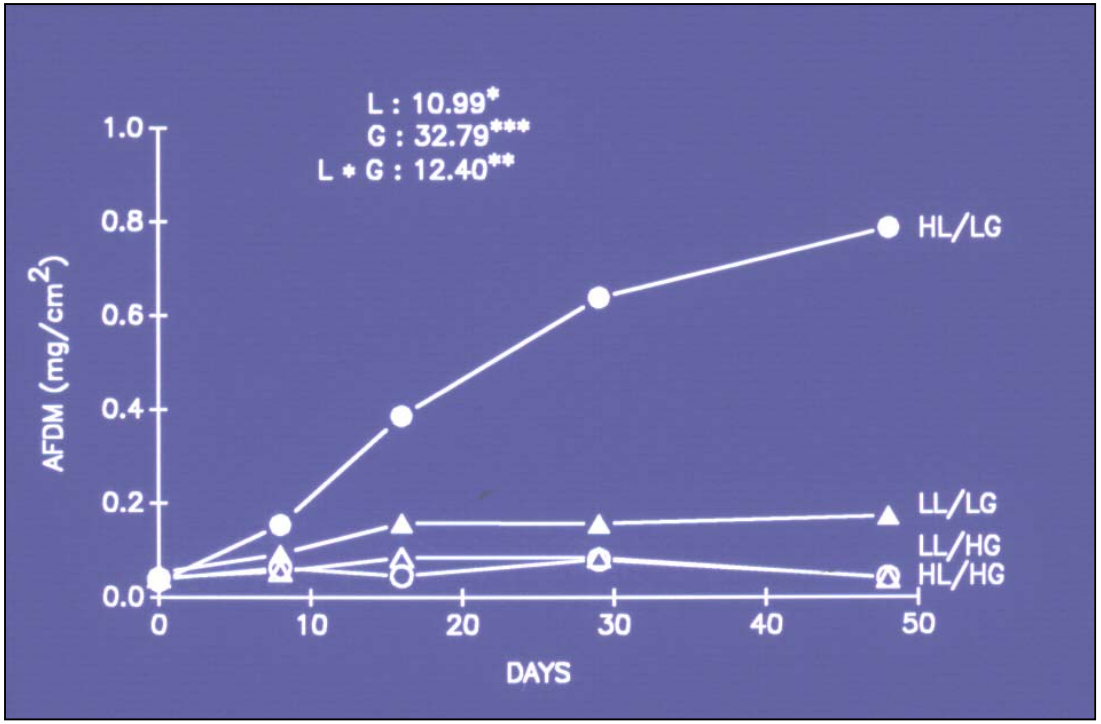
- Pros: accounts for physiological state of algae, integrates environmental conditions, deals only with autotrophs (unlike oxygen)

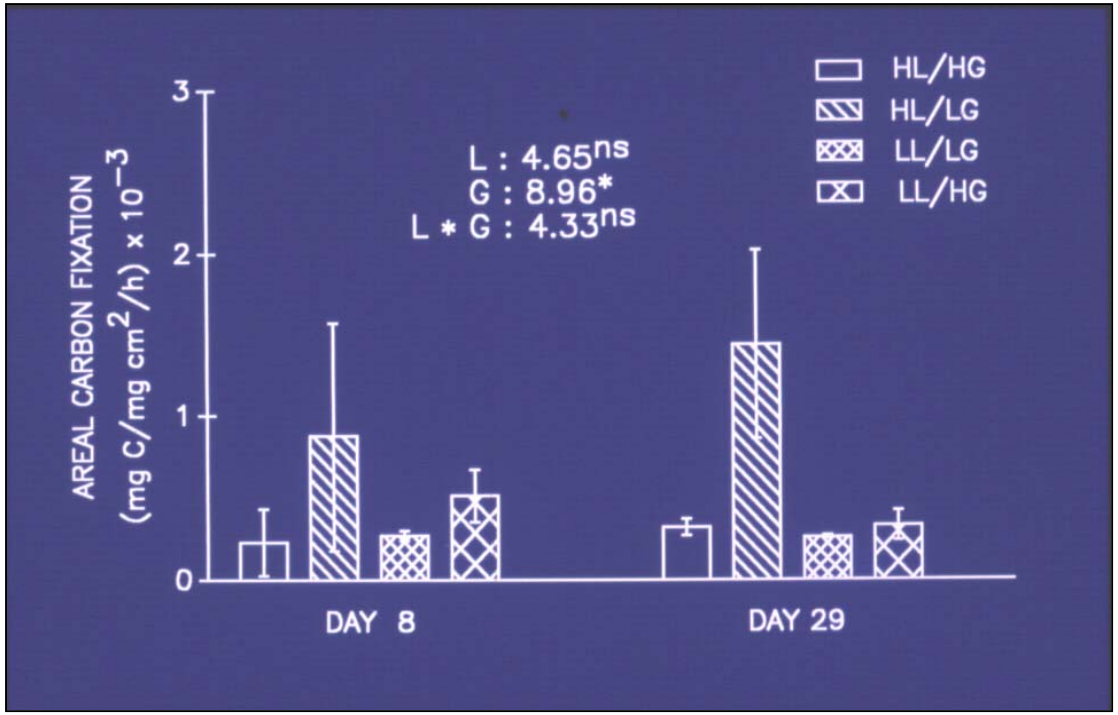
- Cons: radioactive material, chambers may not be representative of ecosystem; time-consuming

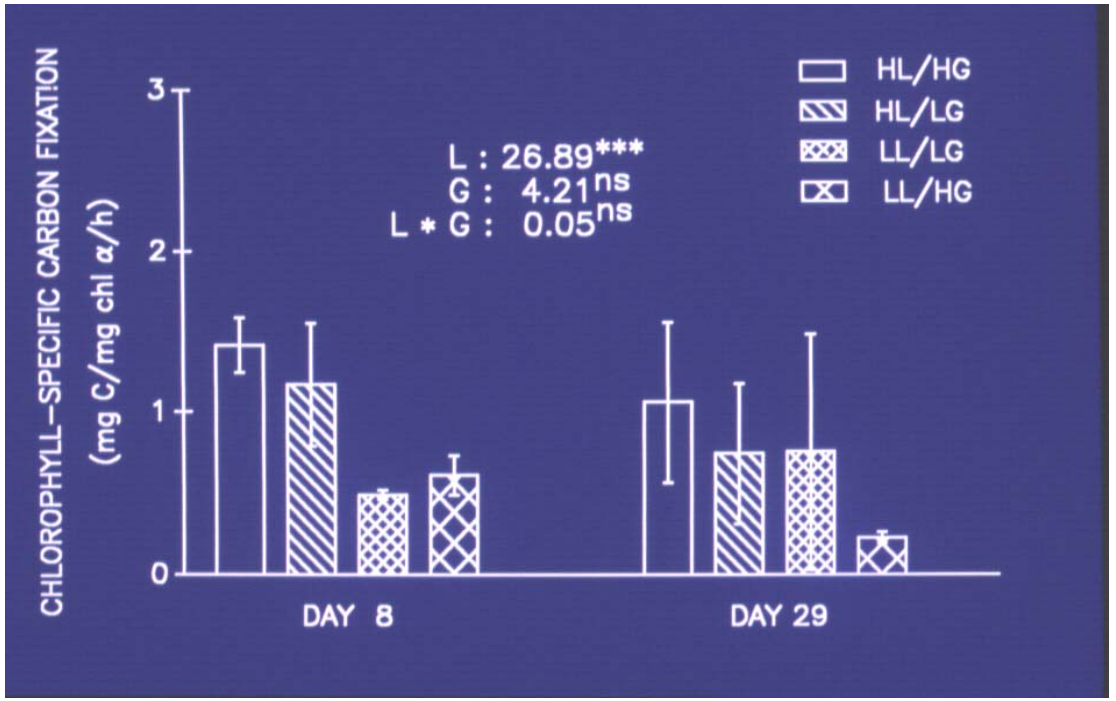
Light Addition

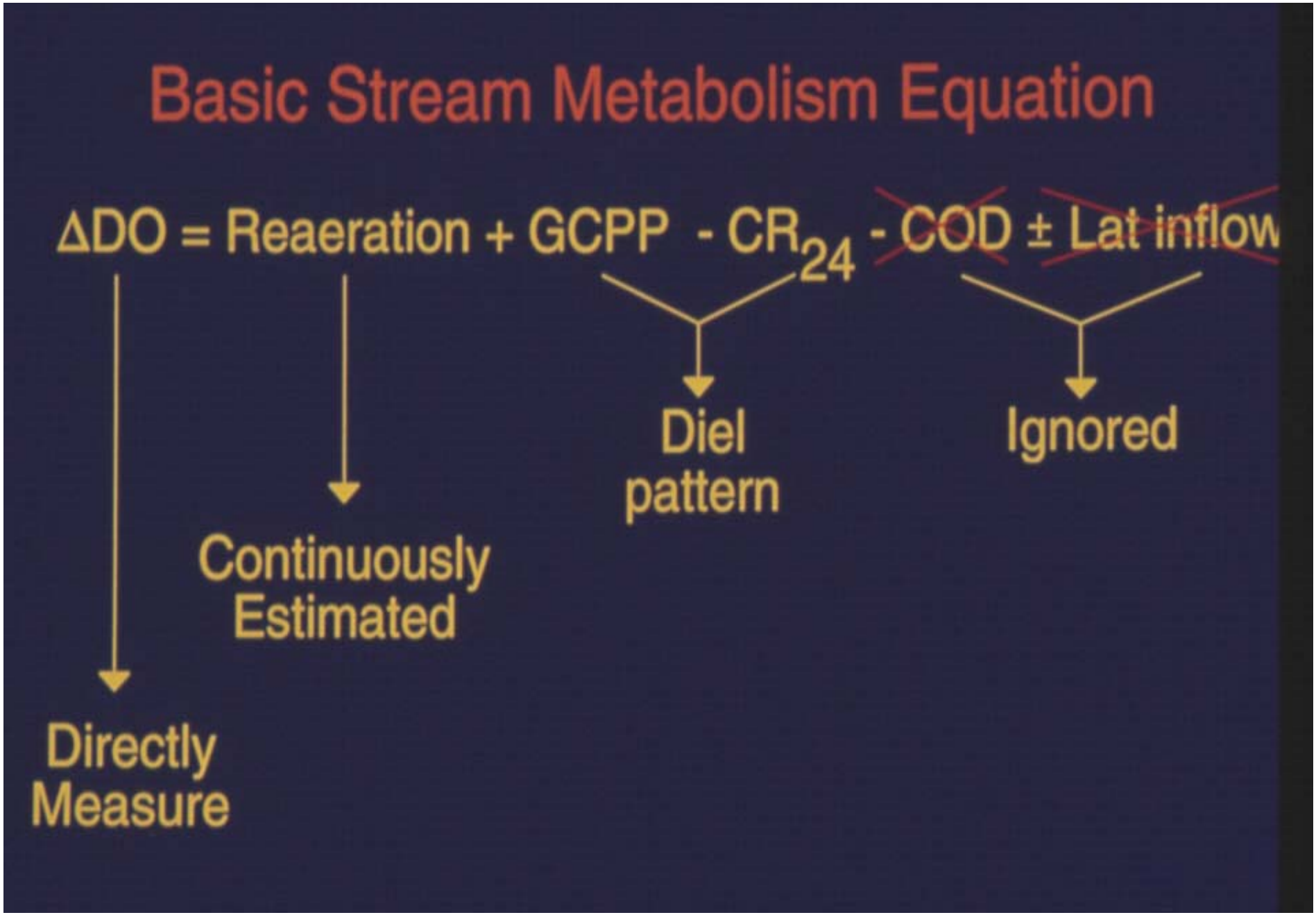
TREATMENTS

	LIGHT LEVEL	GRAZERS	ACRONYM
1)	HIGH	AMBIENT (HIGH)	HL, HG
2)	HIGH	EXCLUDED (LOW)	HL, LG
3)	LOW	AMBIENT (HIGH)	LL, HG
4)	LOW	EXCLUDED (LOW)	LL, LG




AREAL CARBON FIXATION
(mg C/mg cm^2/h) x 10^{-3}
3
2
1
0
L : 4.65^{ns}
G : 8.96*
L * G : 4.33^{ns}
HL/HG
HL/LG
LL/LG
LL/HG
DAY 8
DAY 29


CHLOROPHYLL-SPECIFIC CARBON FIXATION
(mg C/mg chl α/h)
3
2
1
0
L : 26.89***
G : 4.21^{ns}
L * G : 0.05^{ns}
HL/HG
HL/LG
LL/LG
LL/HG
DAY 8
DAY 29

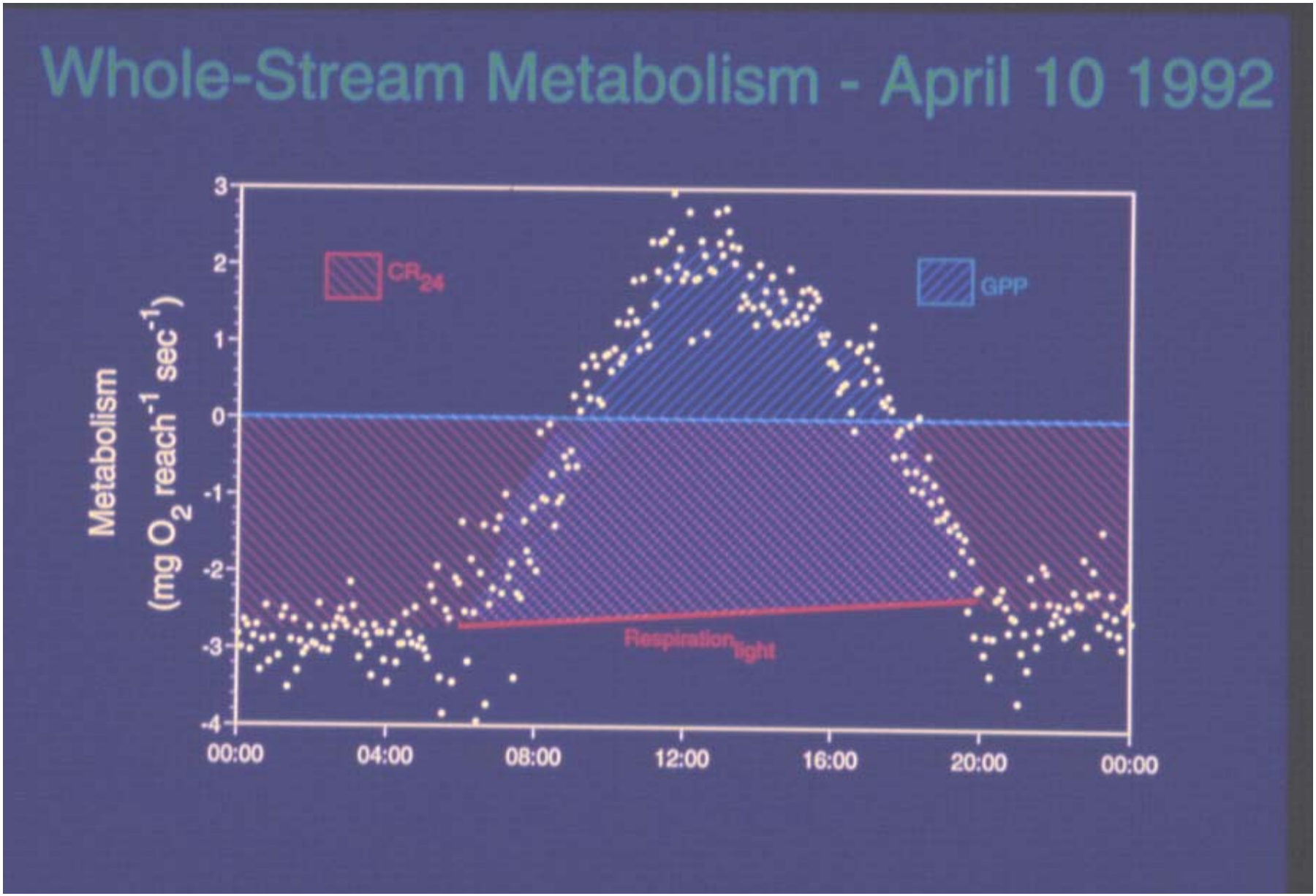

Basic Stream Metabolism Equation
ΔDO = Reaeration + GCPP - CR24 - COD ± Lat inflow
Directly Measure
Continuously Estimated
Diel pattern
Ignored

Reaeration Coefficient Determination

1) Conservative tracer addition:
   - NaCl solution injected by peristaltic pump to increase stream specific conductance
   - Used to calculate travel time and % lateral inflow (dilution)

2) Volatile tracer injection:
   - Propane injected at ~ 4 psi, sampled in glass syringes, and measured by gas chromatography
   - Used to calculate air-water gas exchange coefficient

Source: Marzolf, Mulholland, and Steinman (1994)

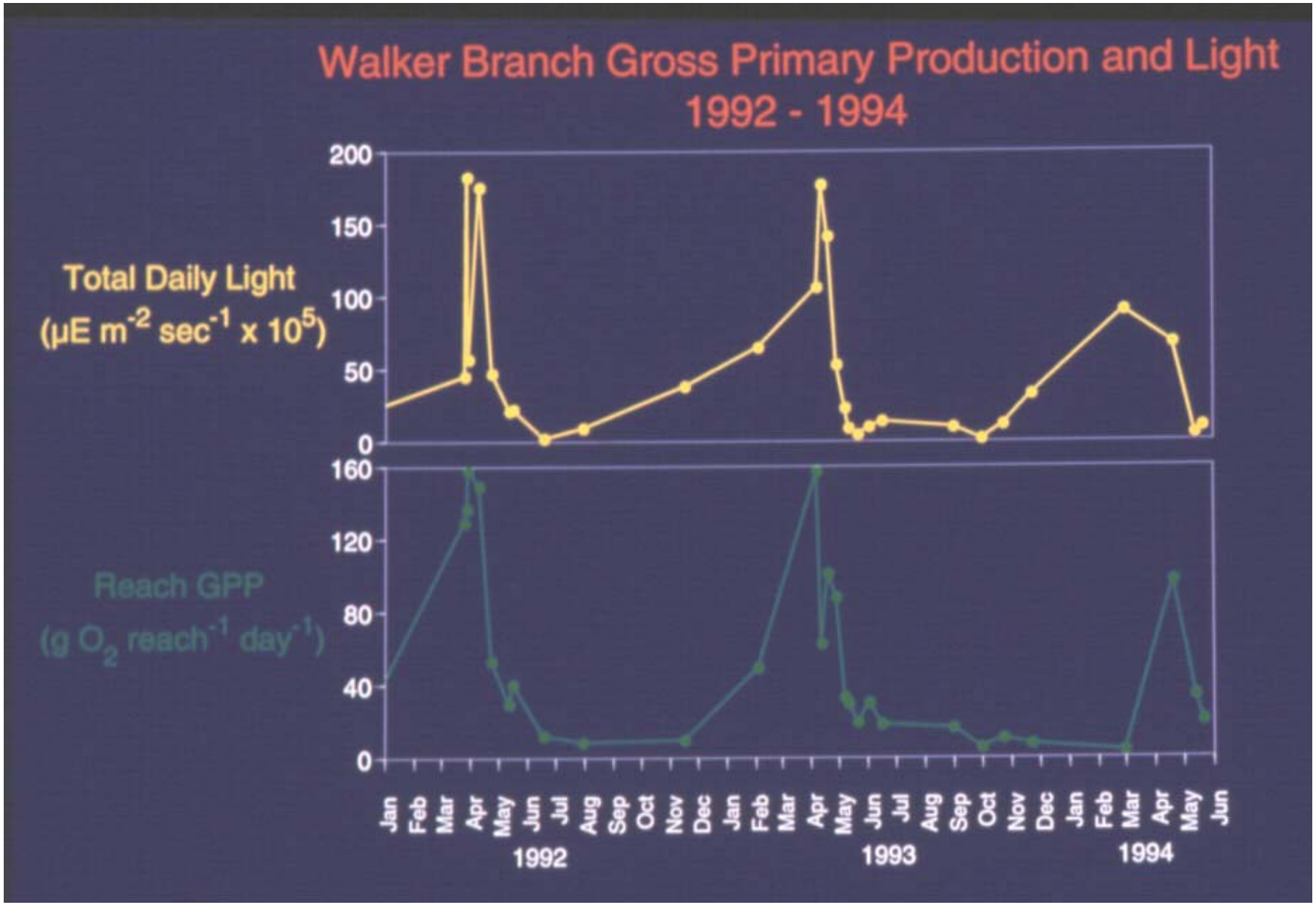


Source: Marzolf, Mulholland, and Steinman (1994)

Whole Stream vs. Chamber Metabolism

Measure (μg O_2/ m^2/s)	Whole-stream (diel)	Chamber (thalweg)	Chamber (backwater)
GPP	21.45	18.69 ± 1.74	13.38 ± 1.32
CR_{24}	-12.43	-4.15 ± 0.73	-3.39 ± 0.52

Source: Marzolf, Mulholland, and Steinman (1994)



Source: Marzolf, Mulholland, and Steinman (1994)

Herbivory Factoids

- Grazer mouthpart morphology will influence ability to graze algae
- Phytoplankton and periphyton, in general, much more vulnerable than macrophytes
- In general, cyanobacteria least preferred of major algal classes
- High grazing pressure may mask high rates of primary productivity

Approaches to Study Grazer Limitation of Primary Producers

- Usually manipulate grazer density and/or type
- Measure community structure, biomass, or metabolic responses to different grazer densities and types
- Information tells you if grazing is constraining growth of autotrophs; if so, nutrient addition will likely not result in increased biomass (but may get ↑PS)

Methods to Assess Herbivore Limitation

Method	Lakes/ ponds	Wadeable streams	Nonwade-able streams
Exclusion/dilution experiments	✓	✓	✓
Addition experiments	✓	✓	✓
Correlation analysis	✓	✓	✓

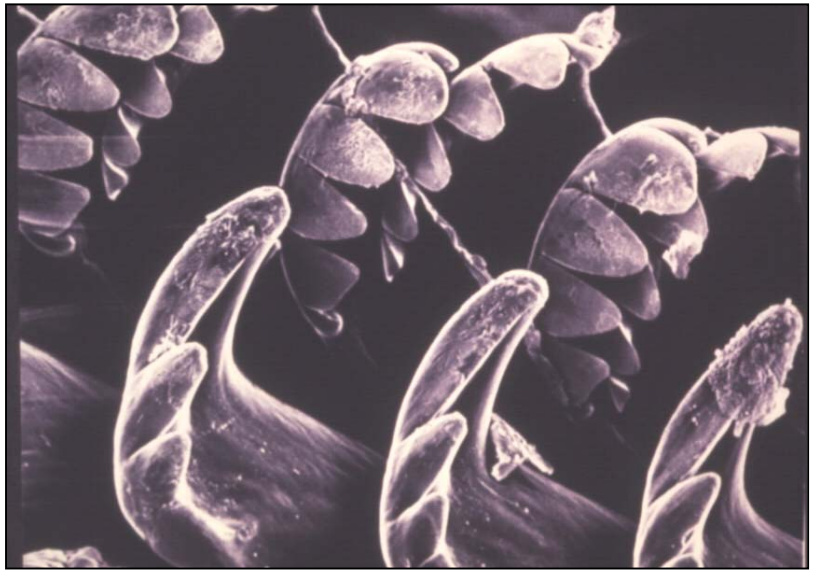
Exclusion/Dilution

- Lakes/Nonwadeable streams:
  - filter zooplankton from water column or sequentially dilute field sample; place filtered/diluted samples in carboys in field or in laboratory setting
- Wadeable streams:
  - exclude benthic grazers from algae by physical, chemical, or electric barriers
  - Strength: determine cause and effect
  - Weakness: time and labor-intensive

Addition

- Lakes/Nonwadeable streams:
  - add zooplankton to field samples; place amended samples in carboys in field or in laboratory setting
- Wadeable streams:
  - add benthic grazers in controlled setting (e.g. experimental channels)
- Strength: determine cause and effect
- Weakness: time and labor-intensive

Grazer Mouthpart Morphology influences algal interaction

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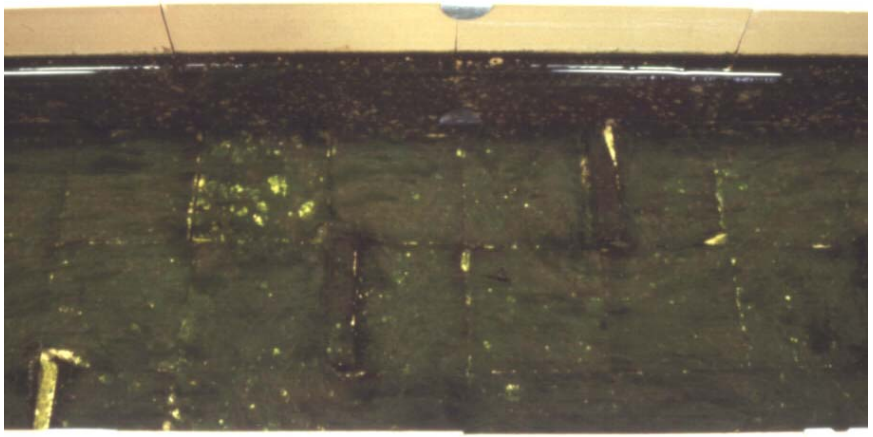
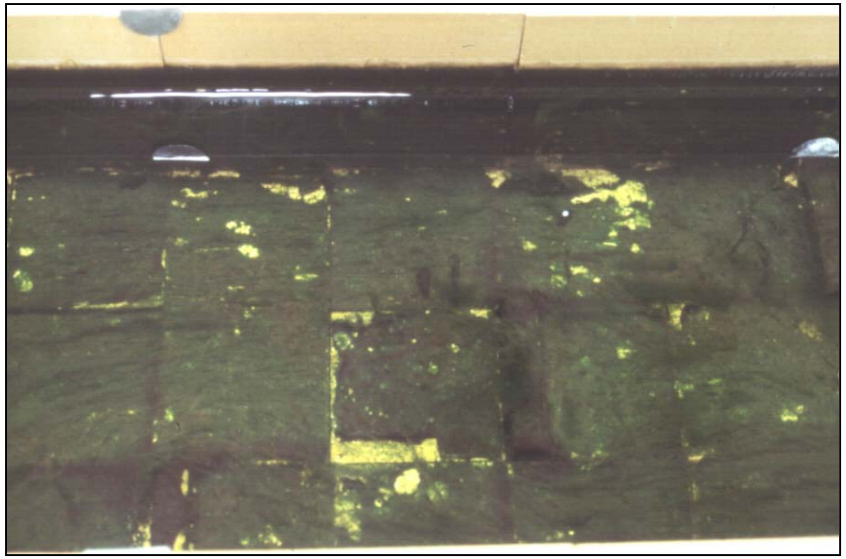
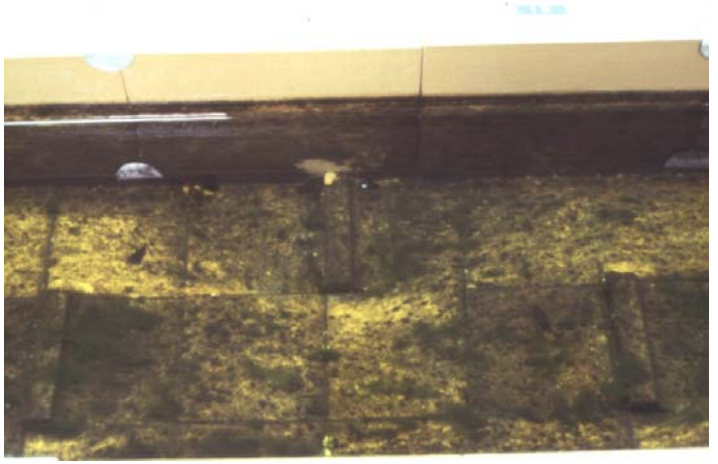
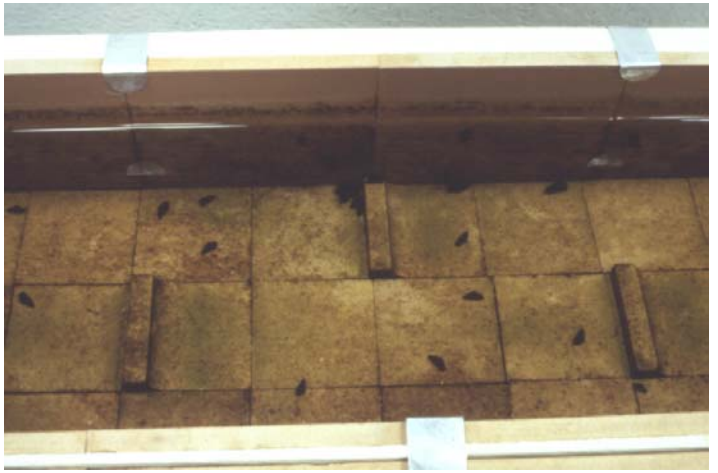
Effect of snail density

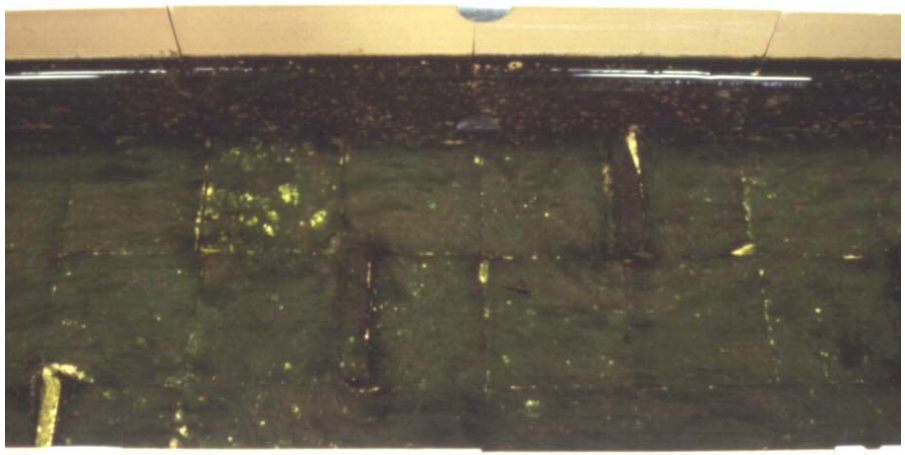


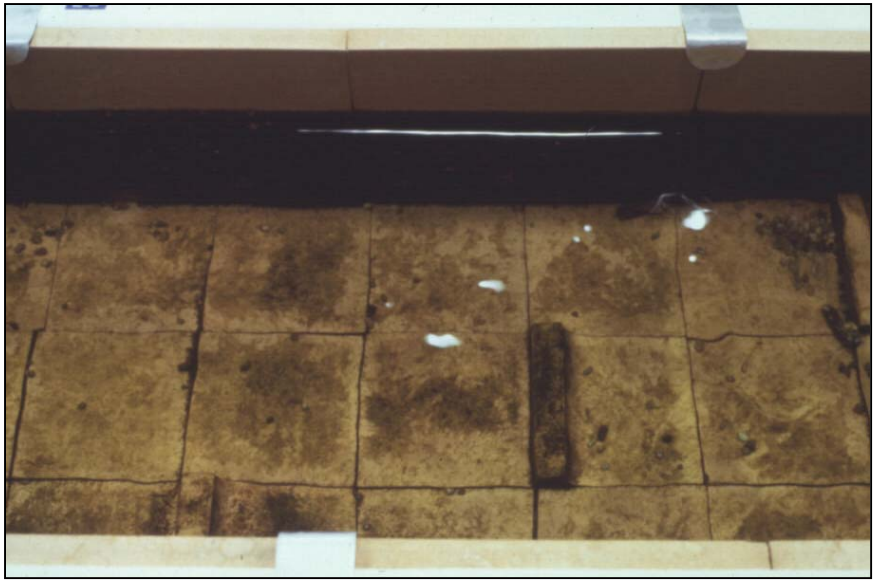


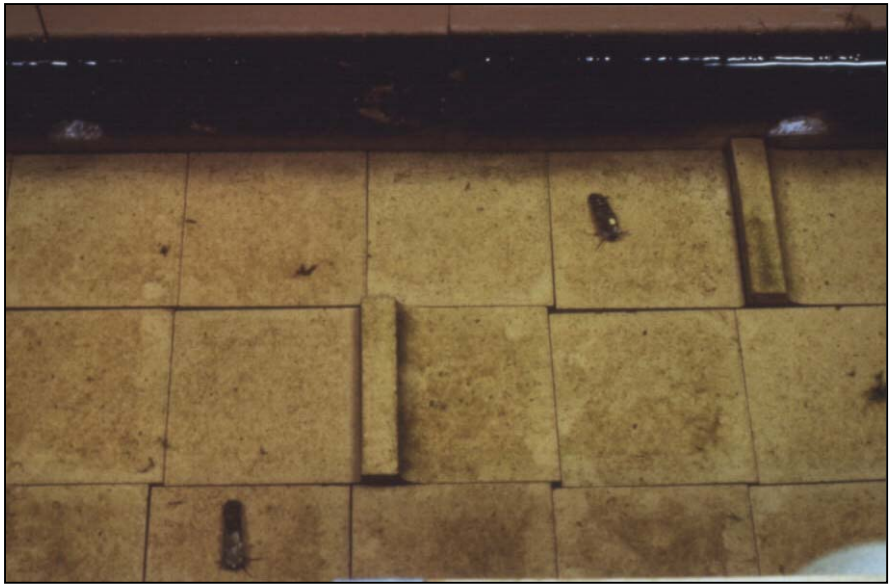




Effect of caddisfly density







Correlation

- All systems:
  - correlate grazer biomass to primary producer biomass

- Strength: use available monitoring data so relatively low time and effort
- Weakness: - cannot determine causation
  - algal-grazer interaction can be complex

Nutrient Factoids

- Phosphorus:
  - essential nutrient; ATP, ADP, nucleic acids, co-enzymes, phospholipids
  - usually ranges from 0.1% to 1.0% of FW algae in nature
- Nitrogen:
  - essential nutrient; proteins, nucleic acids, pigments
  - usually ranges from 0.8% to 11% of FW algae in nature
- Silicon:
  - essential component of diatom frustules (cell walls)
  - usually ranges from 10% to 30% of diatom dry mass

Nutrient Forms

- N and P exist in several forms:

1) Inorganic vs. Organic species
   - Nitrogen: NH_4, NO_2, NO_3 vs. urea, amino acids
   - Phosphorus: PO_4 vs. ADP, ATP

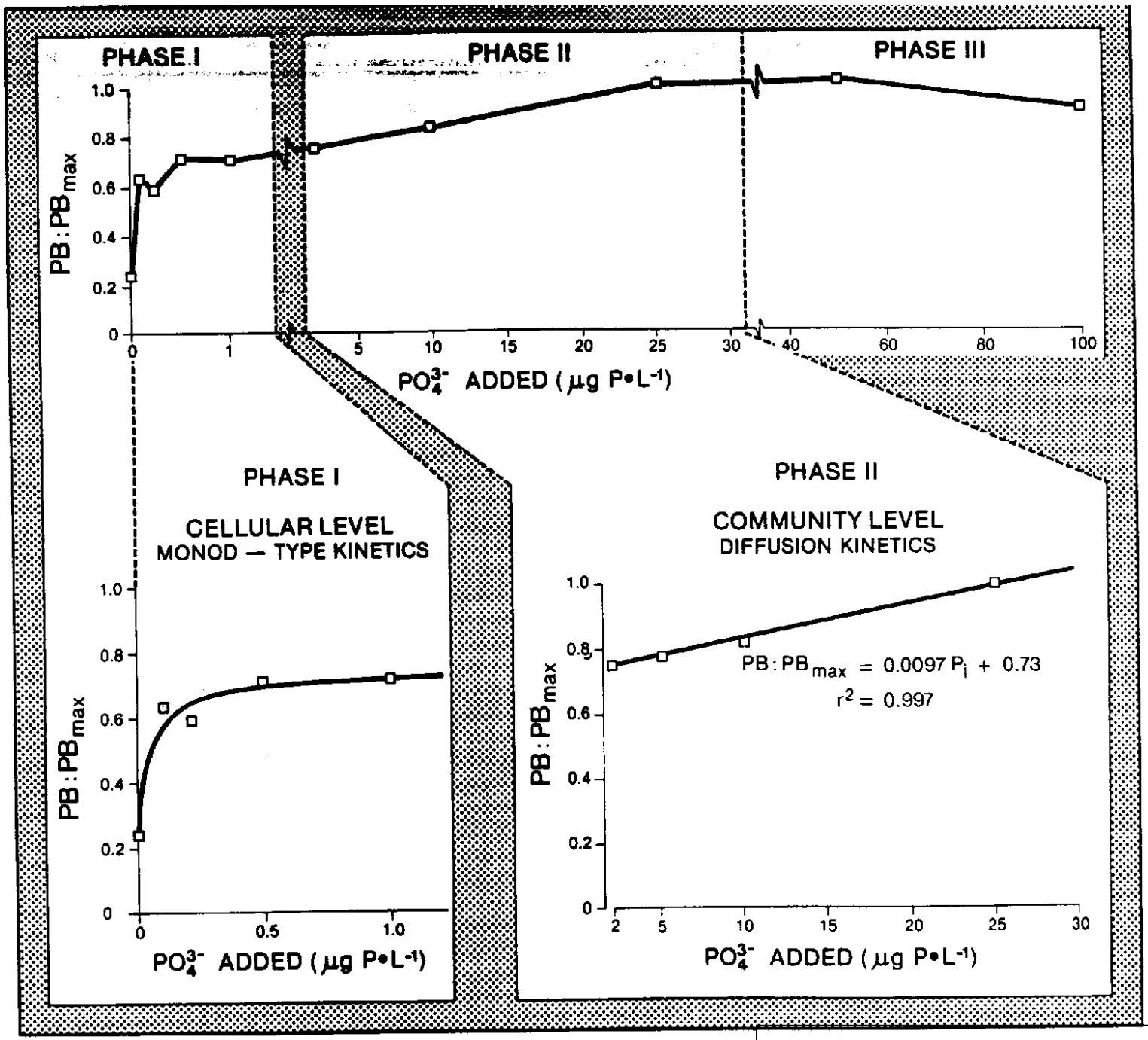
2) Particulate vs. Dissolved (passes through a 0.45 µm membrane filter)
   - Nitrogen: DIN and DON vs PN (microbial cells)
   - Phosphorus: DIP and DOP vs PP (microbes)

Approaches to Study Nutrient Limitation of Primary Producers

- Measure nutrient concentrations in water or in autotroph tissue and compare to literature values
- Measure physiological attribute of autotrophs that is sensitive to nutrient concentration
- Add nutrients to ecosystem or enclosures and measure autotrophic response
- Information can tell you whether or not nutrients are limiting growth of autotrophs, and if so, which nutrient(s) is (are) limiting

Responses of Primary Producers to Added Nutrients

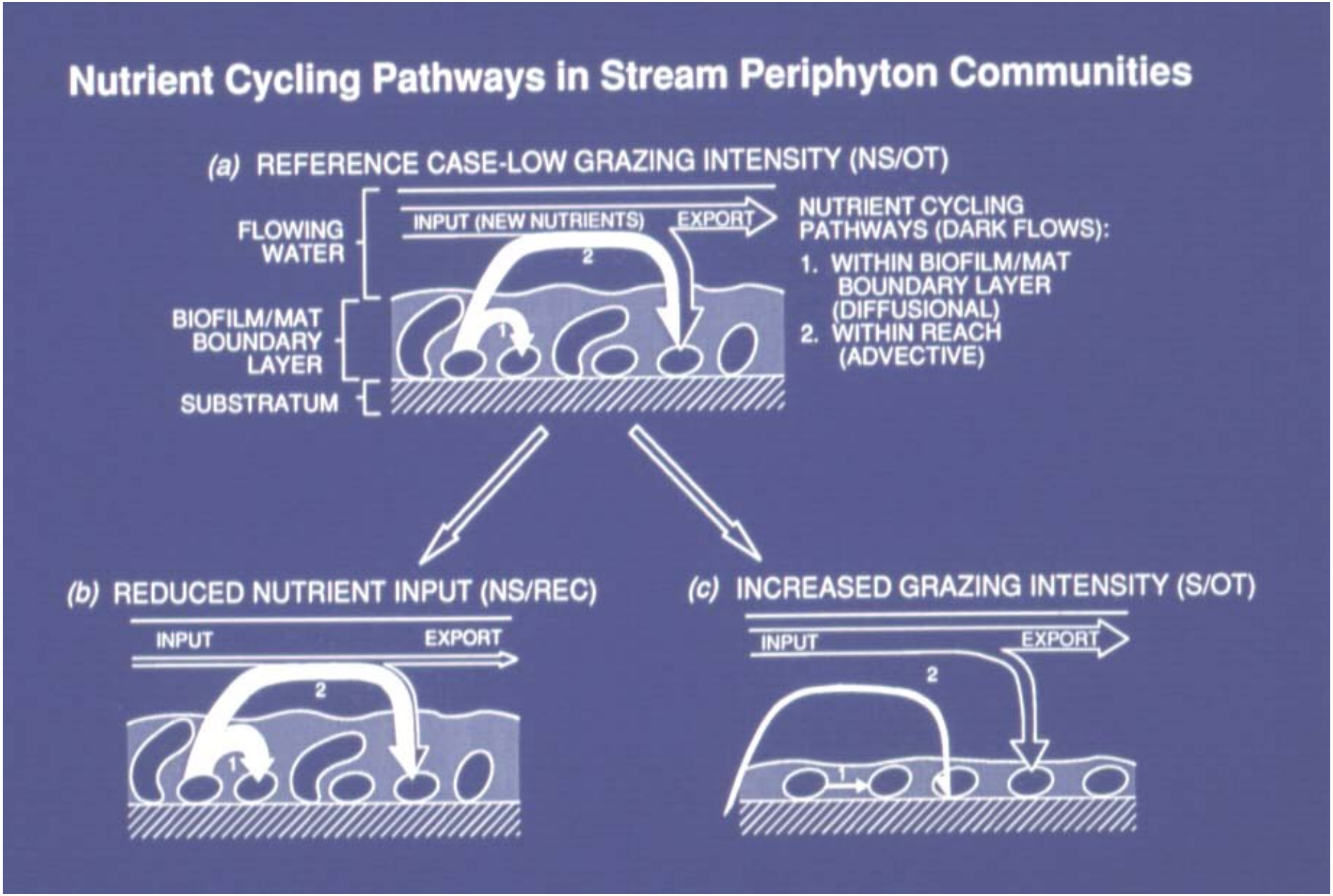
1) Biomass
   - **Increasing the concentration of a limiting nutrient can result in an increase of autotrophic biomass**



Source: Bothwell 1989

Responses of Primary Producers to Added Nutrients

1) Biomass
   - Increasing the concentration of a limiting nutrient can result in an increase of autotrophic biomass
   - **However, algal biomass increase can be masked if another resource is more limiting (e.g. light) or consumptive capacity of grazers exceeds the productive capacity of algae**



Source: Mulholland et al. 1991

Responses of Primary Producers to Added Nutrients

1) Biomass
   - Increasing the concentration of a limiting nutrient can result in an increase of autotrophic biomass
   - However, algal biomass increase can be masked if another resource is more limiting (e.g. light) or consumptive capacity of grazers exceeds the productive capacity of algae
   - **Rooted macrophyte biomass increase may be masked because they obtain nutrients from sediments, which may not reflect water column conditions**

Responses of Primary Producers to Added Nutrients

2) Primary Productivity

- Increasing the concentration of a limiting nutrient can result in increased primary productivity

- Can measure C-fixation, O_2 evolution, or P-I curves

- However, photosynthesis is highly dependent on prior light history, temperature, dissolved inorganic carbon concentration in water, and spp. composition

Responses of Primary Producers to Added Nutrients

3) Species Composition
   - increasing nutrient concentration can result in a change in species composition
   - Phytoplankton: often cyanobacteria, esp. when N:P molar ratio is < 20:1 (Smith et al. 1982)
   - Periphyton: often filamentous green algae (*Cladophora*)
   - Macrophytes: most work done in Europe, with some indicator species (*Nuphar lutea, Potamogeton crispus, P. pectinatus, Sagittaria sagittifolia*)

Methods to Assess Nutrient Limitation

Method	Lakes/ Ponds	Wadeable streams	Nonwade-able streams
Water concent'n	✓	✓	✓
Nutrient Addt'n:			
1) Slug/Drip addn's	✓	✓	✓
2) Enclosure addn's	✓	**X**	✓
3) Nutrient-diffusing substrates	✓ (shoreline/ shallow)	✓	✓ (shoreline/ shallow)

Methods to Assess Nutrient Limitation (con'd):

Method	Lakes/ Ponds	Wadeable streams	Nonwade-able streams
Stoichiometry	✓	✓	✓
Physiological response	✓	✓	✓
Correlation Analysis	✓	✓	✓

Nutrient Concentration Thresholds

- Do you use dissolved or total concentrations?
  - low dissolved concentrations may be due to high uptake rates
  - high total concentrations may reflect biologically unavailable nutrients in water
- Thresholds are site-specific; general guidelines

System	TP (µg/L)	TN (µg/L)	DIP (µg/L)	DIN (µg/L)
Lakes/ponds	30-40	250-300	N/A	N/A
Rivers/streams	~20	~300	~10	~100

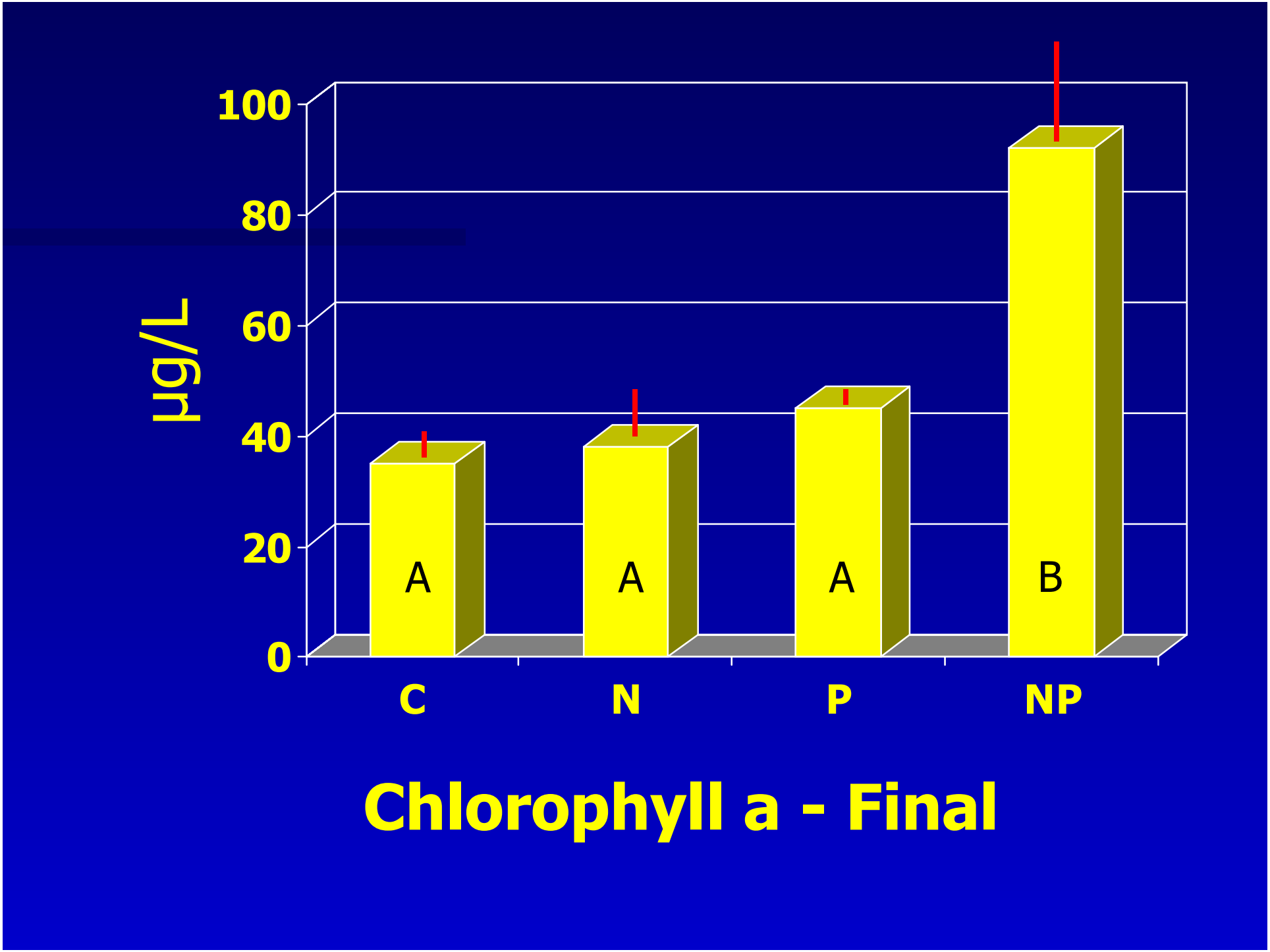
Sources: OECD (1992); Van Nieuwenhuyse and Jones (1996)

Slug or Drip Additions

- Lakes:
  - add slug of nutrient mixture to water column and track algal growth
- Rivers and streams:
  - drip or pump nutrients into stream by peristaltic pump or Mariotte bottle
  - usually add a conservative tracer (e.g. Cl or Br) to track dilution and velocity
- Pros: conducted in natural environment
- Cons: time-consuming, may saturate system

Enclosure Additions

- Lakes:
  - Fill carboys (flasks) with sample from water column, add nutrients to carboys, and deploy back in field (laboratory)
- Nonwadeable Rivers:
  - drip or pump nutrients into stream by peristaltic pump or Mariotte bottle
  - usually add a conservative tracer (e.g. Cl or Br) to track dilution and velocity
- Pros: replication, multiple treatments
- Cons: artifacts of containment, time-consuming



Chlorophyll a - Final

Nutrient-Diffusing Substrates

- All systems:
  - Fill flower pots or other diffusive substrate with agar-enriched nutrients
  - Sample periphyton over time

- Pros: replication, multiple treatments (different nutrients), ease

- Cons: nutrients for periphyton usually come from water column—not substrate, time-intensive (20-30 d), measures net growth, must ensure release is constant

Slide courtesy of Dean DeNicola

Stoichiometry

- All systems:
  - Analyze elemental ratios of autotrophs
  - Compare with literature values
- C:N:P Ratios (molar):
  - FW benthic algae: 158:18:1 (Kahlert 1998)
  - Marine benthic algae: 119:17:1 (Hillebrand and Sommer 1999)
  - Marine phytoplankton: 106:16:1 (Redfield 1958)
  - FW macrophytes: ~1.3% N dry mass; ~0.13% P dry mass (Gerloff and Krombholz 1966)
  - Pros: does not require experimentation
  - Cons: not species-specific; time-consuming

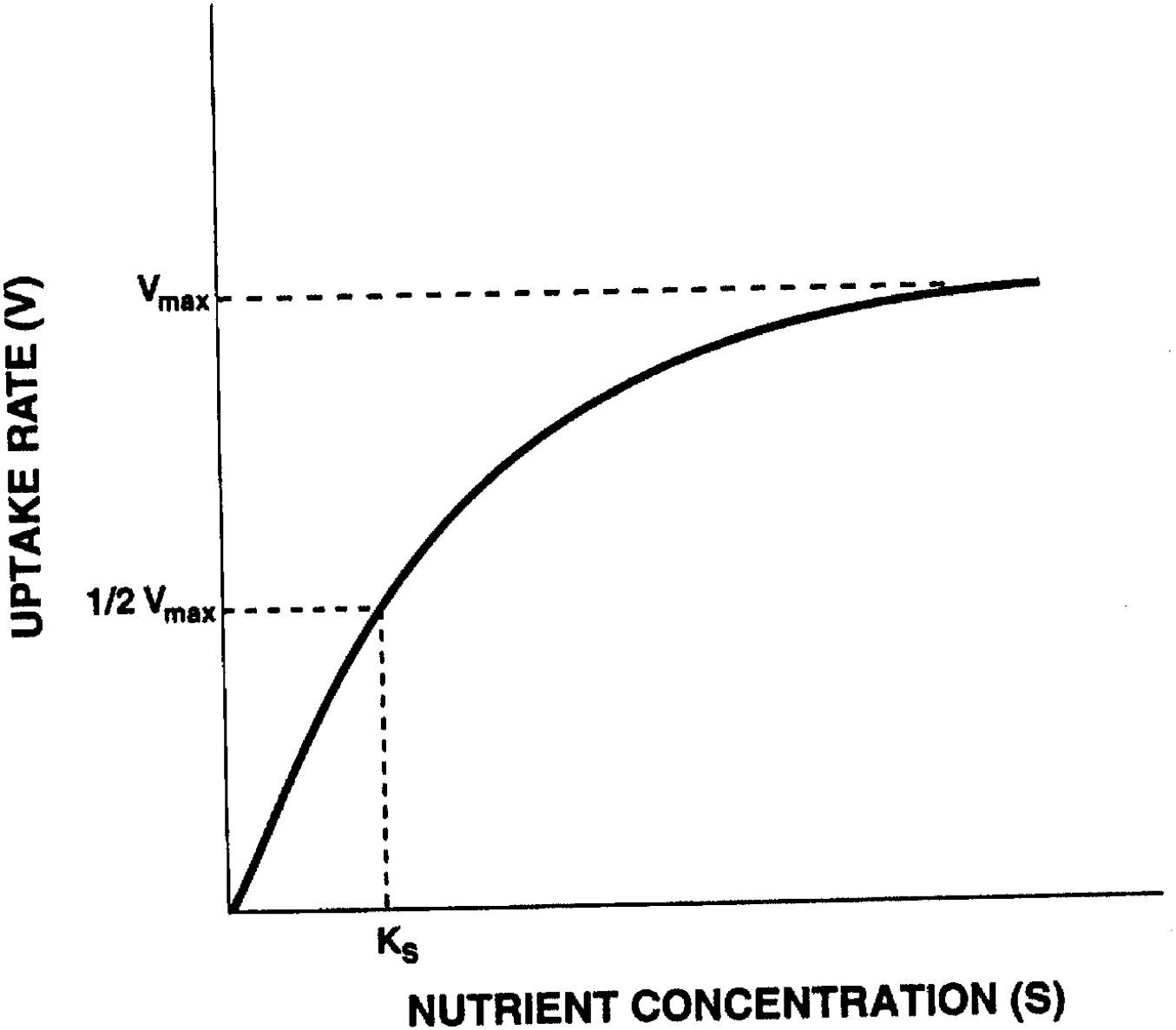
Stoichiometry

- Phosphorus Deficiency:

Algal Type	C:P (molar)	N:P (molar)
FW Phytoplankton (Hecky et al. 1993)	>129	>22
FW Benthic Algae (Kahlert 1998)	369	32

Physiological Response

- All systems:
  1) Analyze Michaelis-Menten kinetics
  - Compare with literature values
- M-M kinetics:
  - grow species under different concentrations of limiting nutrient and measure kinetics
  - V_{max}: maximum nutrient uptake rate
  - K_s: half-saturation constant (nut. concentration at which nutrient uptake is ½ V_{max})
- Pros: sensitive, info on competitive ability
- Cons: varies by species so community-level response hard to interpret, time-consuming



Source: Steinman and Mulholland (1996)

Physiological Response

- All systems:
  2) Analyze enzyme kinetics
  - Compare with literature values

- Phosphatase:
  - hydrolyzes phosphate ester bonds, releasing orthophosphate (PO_4) from organic P compounds
  - alkaline phosphatase most common in FW
  - As inorganic P ↓, PA usually ↑

- Pros: sensitive, does not require manipulation

- Cons: not species-specific, other phosphatases may be important, time-consuming, only good for P

Phosphatase Activity

P Deficiency	PA (mmol/mg Chl *a*/ hr)
Moderate	> 0.003
Severe	> 0.005

Source: Healey and Hendzel 1979

Correlation

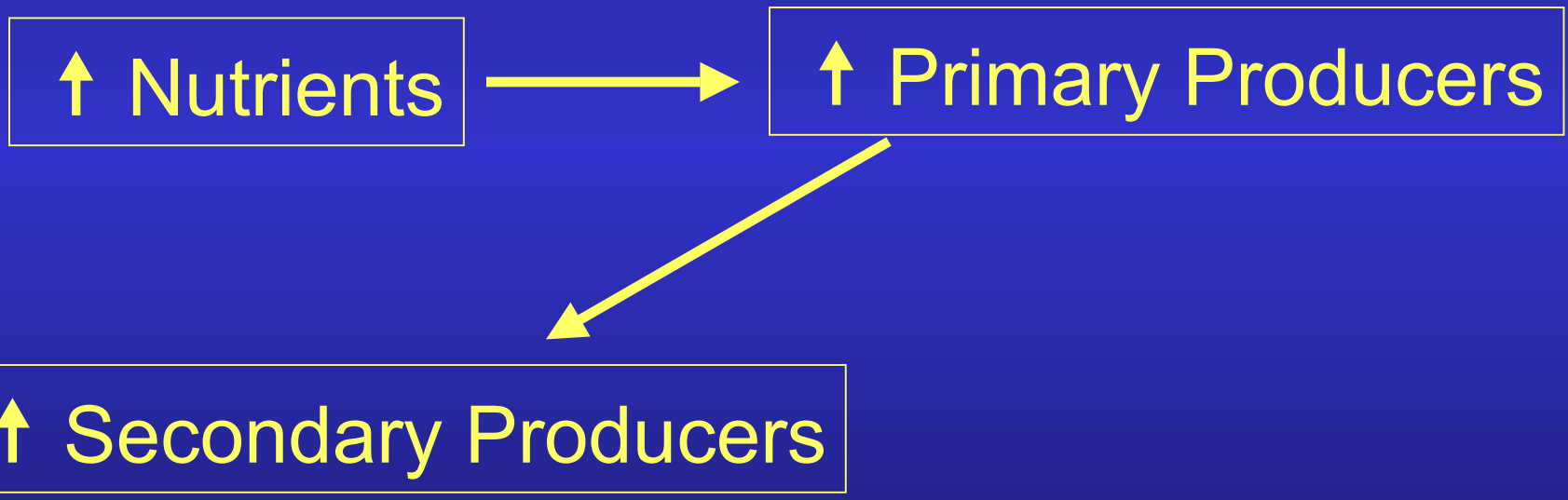
- All systems:
  - correlate nutrient concentration to primary producer biomass

- Strength: use available monitoring data so relatively low time and effort
- Weakness: - cannot determine causation
  - algal-nutrient interaction can be complex

Food Web Implications

- Bottom-up vs. Top-down

  1) Bottom-up:

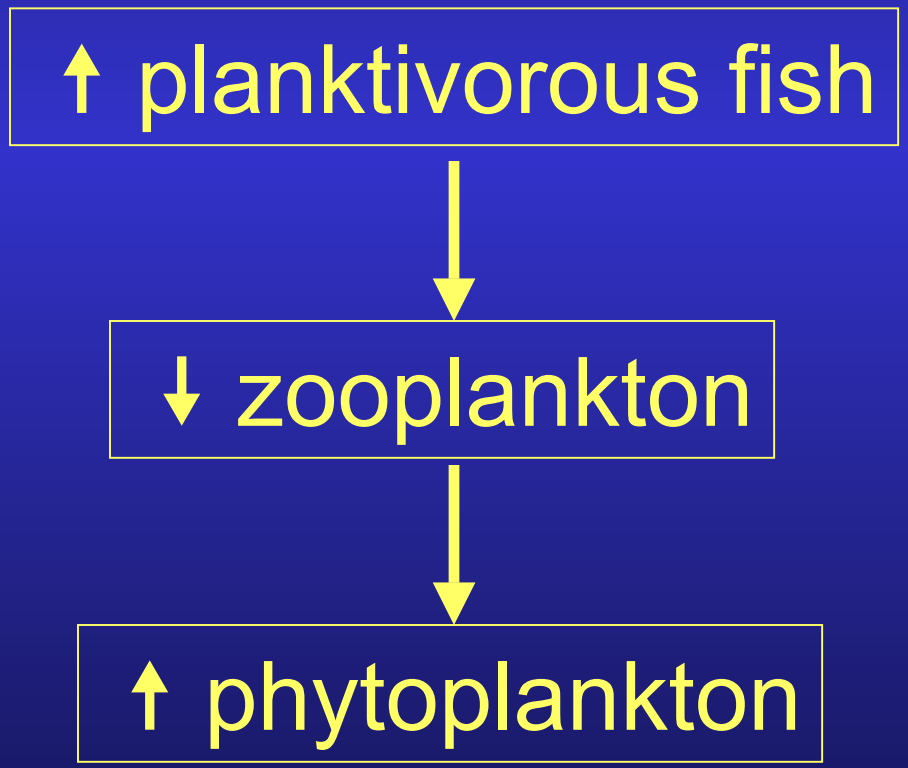


(cf. Carpenter et al. 1985)

Food Web Implications

- Bottom-up vs. Top-down

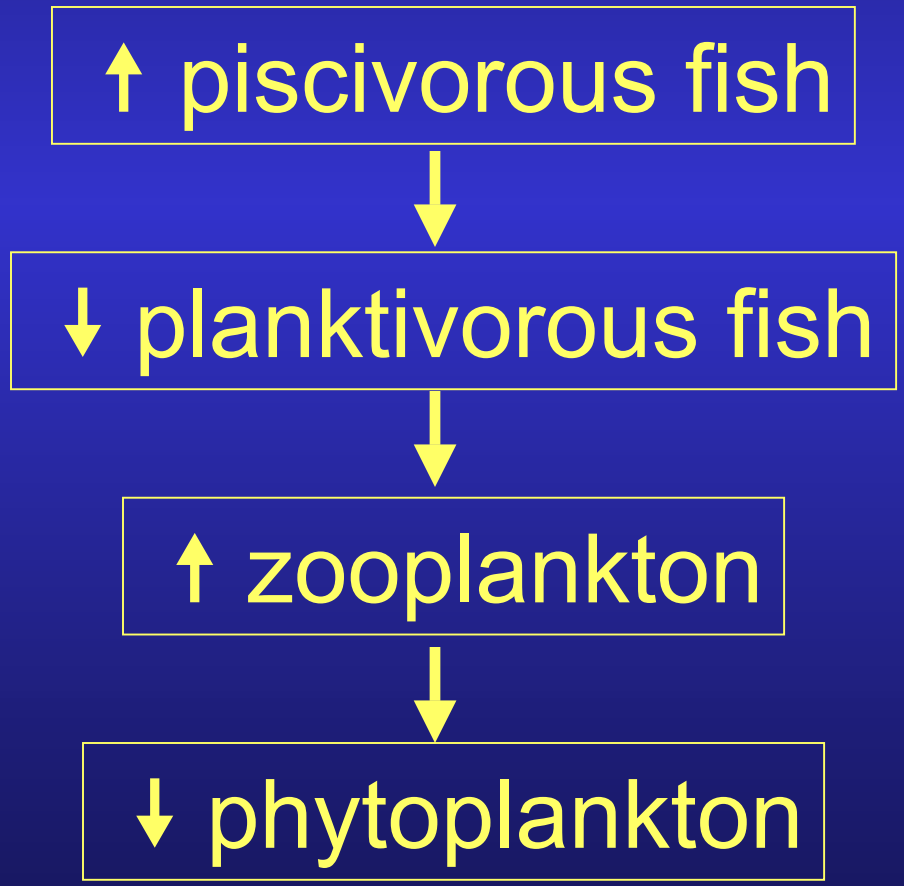
  2a) Top-down (odd # trophic levels):

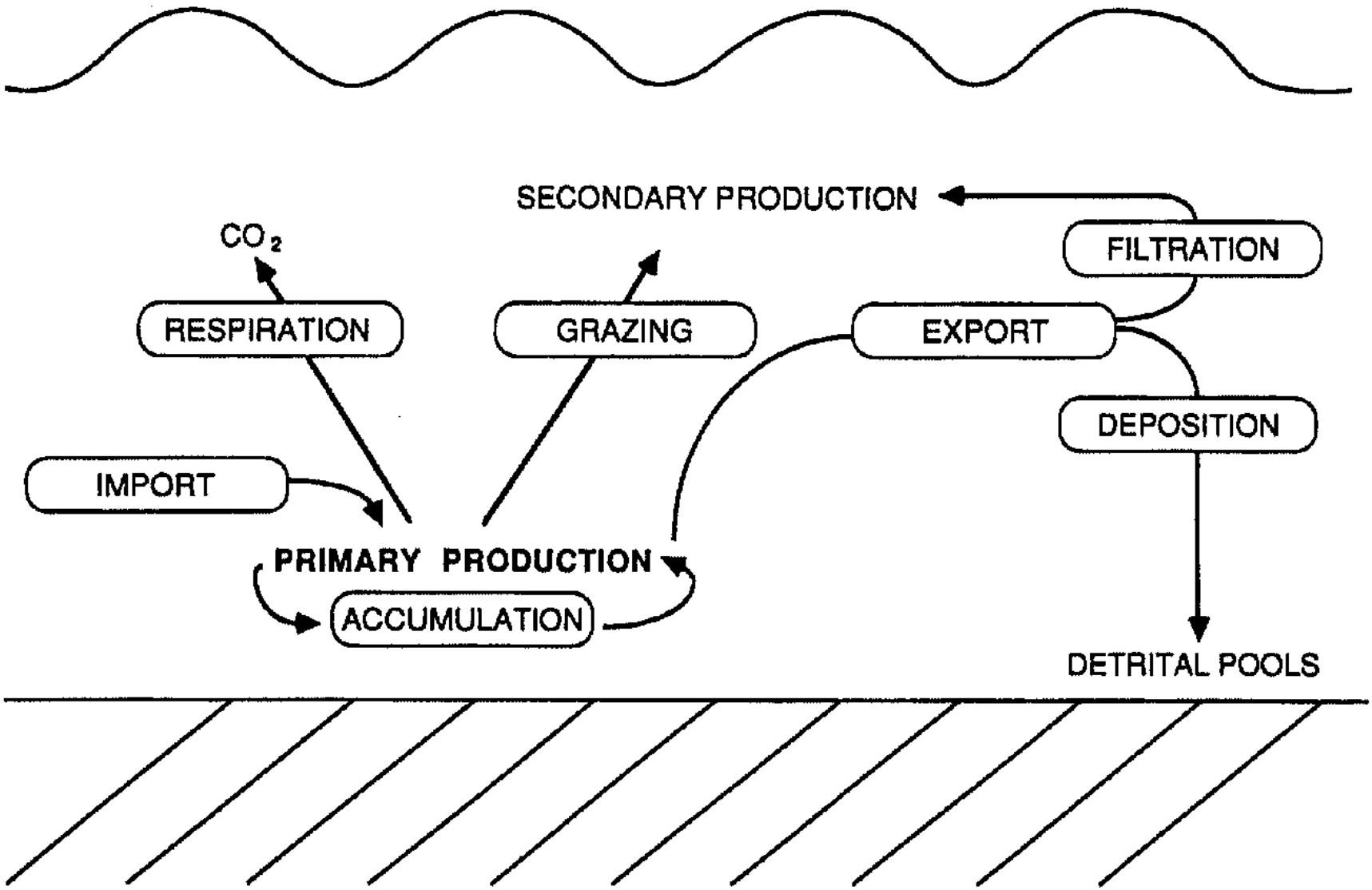


Food Web Implications

- Bottom-up vs. Top-down

  2b) Top-down (even # trophic levels):





Source: Lamberti 1996

Summary

- Primary producers are at the base of the food web and provide energy both directly (herbivory) and indirectly (detritus)
- Primary producers can be measured in terms of biomass, metabolism, or community structure
- Nutrients, light, herbivory, and temperature all influence primary producers, often in complex ways due to their interactions
- There are many ways to assess the factors limiting primary producers, and each has its own strengths and weaknesses

References

Bothwell, M.L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Can. J. Fish. Aquat. Sci. 46: 1293-1301.

Carpenter, S.R., J.F. Kitchell, and J.R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience 35: 634-639.

Davies-Colley, R.J, W.N. Vant, and D.G. Smith. 1993. *Colour and Clarity of Natural Waters*. Ellis Horwood.

Dodds, W.K., V.H. Smith, and B. Zander. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: A case study of the Clark Fork River. Water Res. 31: 1738-1750.

Dodds, W.K. and E.B. Welch. 2000. Establishing nutrient criteria in streams. J. No. Am. Benthol. Soc. 19: 186-196.

Gerloff, G.C. and P.H. Krombholz. 1966. Tissue analysis as a measure of nutrient Availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. 11: 529-537.

Healey, F.P. and L.L. Hendzel. 1979. Fluorometric measurement of alkaline phosphatase activity in algae. Freshwat. Biol. 9: 429-439.

Hecky, R.E., P. Campbell, and L.L. Hendzel. 1993. The stoichiometry of carbon, Nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnol. Oceanogr. 38: 709-724.

References (continued)

Hill, W.R. 1996. Effects of light. Pages 121-148 In: *Algal Ecology*. Stevenson, R.J., Bothwell, M.L. and R.L. Lowe (editors). Academic Press.

Hillebrand, H. and U. Sommer. 1999. The nutrient stoichiometry of benthic microalgal Growth: Redfield proportions are optimal. Limnol. Oceanogr. 44: 440-446.

Kahlert, M. 1998. C:N:P ratios of freshwater benthic algae. Arch. Hydrobiol., Spec. Iss. Adv. Limnol. 51: 105-114.

Kirk, J.T.O. 1986. *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge Univ. Press.

Lamberti, G.A. 1996. Role of periphyton in benthic food webs. Pages 533-572 In: *Algal Ecology*. Stevenson, R.J., Bothwell, M.L. and R.L. Lowe (editors). Academic Press.

Marzolf, E.R., P.J. Mulholland, and A.D. Steinman 1994. Improvements to the diurnal upstream-downstream dissolved oxygen technique for determining whole-stream metabolism in small streams. Can. J. Fish. Aquat. Sci. 51: 1591-1599.

Mulholland, P.J., A.D. Steinman, A.V. Palumbo, J.W. Elwood, and D.B. Kirschtel. 1991. Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. Ecology 72: 966-982.

OECD. 1982. *Eutrophication of Waters: Monitoring, Assessment and Control*. OECD, Paris.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. Am. Sci. 46: 205-221.

References (continued)

Smith, V.H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: an empirical and theoretical analysis. Limnol. Oceanogr. 27: 1101-1112.

Steinman, A.D. 1992. Does an increase in irradiance influence periphyton in a heavily grazed woodland stream? Oecologia 91: 163-170.

Steinman, A.D. 1996. Effects of grazers on freshwater benthic algae. Pages 341-373 In: *Algal Ecology*. Stevenson, R.J., Bothwell, M.L. and R.L. Lowe (editors). Academic Press.

Steinman, A.D. and G.A. Lamberti. 1996. Biomass and pigments of benthic algae. Pages 295-314 In: Methods in Stream Ecology. Hauer, F.R. and G.A. Lamberti (editors). Academic Press.

Steinman, A.D., R.H. Meeker, A.J. Rodusky, W.P. Davis, and S.-J. Hwang. 1997. Ecological properties of charophytes in a large subtropical lake. J. No. Am. Benthol. Soc. 16: 781-793.

USEPA. 2000. *Nutrient Criteria Technical Guidance Manual. Rivers and Streams*. Office of Water. EPA-822-B-00-002.

Van Nieuwenhuyse, E.E. and J.R. Jones. 1996. Phosphorus-chlorophyll relationship In temperate streams and its variation with stream catchment area. Can. J. Fish. Aquat. Sci. 53: 99-105.